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The Intracytoplasmic Sperm Injection (ICSI) technique in an infertile man with Hepatitis-B Virus (HBV) infection: a case report

I Gusti Ngurah Pramesemara^{1,2*}, Zakiyatul Faizah³, Ninik Darsini³

ABSTRACT

Background: Several studies have suggested a relationship between chronic infection of the Human Hepatitis-B Virus (HBV) and infertility in men. HBV infection causes instability of spermatozoa chromosomes, interferes the parameters of semen analysis, and have risks to infecting fetus or embryo. In order to prevent the HBV infection, the Intracytoplasmic Sperm Injection (ICSI) procedure has been established. This case study aims to evaluate the ICSI technique conducted to an infertile man with HBV infection.

Case Description: We reported a case of a 42-year-old infertile male who had primary infertility for 11 years with positive HBsAg.

Patients were given *Tribulus terrestris* and an antioxidant also referred to an internist colleague for the definitive treatment of Hepatitis-B. Then the patient and his wife were prepared to undergo an In-Vitro Fertilization (IVF) procedure by washing spermatozoa and ICSI. Routine evaluation for 15 days post-ICSI for the first time did not show biochemical signs of pregnancy in wives with negative β -hCG.

Conclusion: It was concluded that the ICSI procedure could be considered in the action of IVF in infertile man with HBV due to no adverse effect recorded.

Keywords: ICSI, IVF, Infertile Man, HBV infection

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¹Andrology Specialist-1 Program, Medical Faculty of Universitas Airlangga, Dokter Soetomo General Hospital, Surabaya, Indonesia

²Andrology and Sexology Department, Medical Faculty of Universitas Udayana, Surabaya, Indonesia

³Medical Biology Department, Medical Faculty of Universitas Airlangga, Surabaya, Indonesia

INTRODUCTION

Human Hepatitis B Virus (HBV) is the main epidemic infectious agent of acute and chronic hepatitis, hepatic cirrhosis and hepatocellular carcinoma.¹ Nearly two billion people worldwide have been infected with HBV and around 350 million people live with chronic infections which have the potential to continue transmission of HBV to other healthy peoples.¹ HBV infection is one of the most severe public health problems, especially in the Asia and Pacific regions.^{1,2} Indonesia as the largest archipelagic country in the world has high endemic HBV-related conditions and it is estimated that there are 1.4 million cases of death each year and associated with chronic HBV infection or hepatocellular carcinoma.³

Although HBV is known as hepadnavirus, it is also found in other extrahepatic tissues, such as the kidneys, parotid glands, ovaries and testes.⁴ Therefore, HBV infection is able to be found in most body fluids from a patient, such as in blood, vaginal secretions, saliva and semen.² The above conditions explain that HBV can be transmitted through sexual contact, especially risky sexual habit, without using condoms or not getting a vaccine.²

Several in-depth studies have succeeded in confirming the presence of Deoxyribonucleic acid (DNA) from HBV in gamete cells (spermatozoa

and oocytes) that can increase chromosomal instability and cause genetic disorders.⁵ Integration of HBV into the spermatozoa chromosome can cause decreases in motility, increases apoptosis and necrosis, and mediates the mutagenic effect, which results in a reduction of male fertility.⁶ Also found impaired density, decreased total number, morphology and viability of spermatozoa in male semen analysis with HBV.⁷ The latest report mentions that HBV infection induces abnormal expressions of IL-17 and IL-18 in semen which harm the functioning capacity of male reproductive organs.⁷

Nowadays there is an increasing number of infertile couples with HBV who wish to undergo assisted reproductive technology (ART), including in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and embryo transfer.⁴ Recent clinical studies suggest that HBV infection is significantly associated with a low ratio of pregnancies after IVF.⁸ Based on the above, HBV screening before ART becomes more frequent in many IVF clinics.⁹

Various efforts have been made to increase the success rate of IVF in infertile couples with HBV.¹ Starting from the development of techniques and protocols to help andrologist or embryologist for safety while working, maintaining the sterility of fertility clinics, patient screening and detection

*Corresponding to:

I Gusti Ngurah Pramesemara;
Andrology and Sexology
Department, Medical Faculty of
Universitas Udayana, Surabaya,
Indonesia;
pramesemara@unud.ac.id

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of material that has danger potential of infection, also the management of HBV treatment including vaccination.¹

Based on those mentioned above, this case report of infertile couples with HBV who undergo ICSI procedure can provide a clear and useful overview, also an insight into undergoing IVF clinical practice.

CASE REPORT

A 42-year-old male and ethnic Timorese from the city of Kupang, Nusa Tenggara Timur (NTT) province came to the Graha Amerta Infertility Clinic of the Dr Soetomo General Hospital Surabaya in March 2018 with complaints of not having children and has been married for 11 years (Table 1). The patient lives with his wife, who is also 42 years old and regularly has sexual intercourse without using contraception. Patients and their wives admitted that they had never received treatments and assisted reproductive technology (ART) efforts before.

Semen analysis of patients in February 2018 obtained volume of 2.0 ml with the viscosity of more than 2 cm, greyish white, distinctive odour, pH 6.0, concentration of 0.1 million/ml and less motility (10% progressive, 10% non-progressive

and 80% immotile), so it was concluded that severe oligoasthenozoospermia (OA) (Table 1). In addition, the results of patient laboratory tests in February 2018 showed HBsAg positive reactive, whereas Anti HCV and anti-HIV negative non-reactive. Hormonal laboratory examination found an increased in FSH 42.86 mIU/ml and testosterone 3.57 ng/ml (Table 1).

The patient's wife showed the results of non-reactive HBsAg, Anti HCV, and anti-HIV laboratory tests. Other hormonal examination from patient's wife showed an AMH value decreased 14 pmol/L, while the values of estrogen, FSH, LH, progesterone, prolactin, TSH and free-T4 were within the normal range.

General physical examination has been carried out and it is concluded that the patient is in normal condition. Specific genitalia physical examination found the volume of the right and left testes each 6 cc with softness consistency. They were followed by palpation, which found the condition of the penis, scrotum, epididymis, vas deferens and secondary sexual signs under normal circumstances (Table 1).

Based on results of the history, physical examination and investigation, the patient was declared to have primary infertility state, being given treatment in the form of Tribulus terrestris and antioxidants for thirty days. Besides, patients were referred to an internist to get appropriate therapy regarding HBV infection. Patients are planned to repeat semen analysis and be prepared for ART.

One month later, the patient underwent a semen analysis with the impression of improved results. It was founded that the volume of ejaculate about 5.5 ml, longer viscosity of more than 2 cm, greyish white, distinctive smell, pH 7.5, concentration 0.15 million/ml, and less motility (34% progressive, 2% non-progressive and 64% immotile). It was concluded that there was still the impression of severe OA (Table 1).

Then ejaculate fluid collection was prepared by simple sperm preparation with 2500 rpm centrifugation for 10 minutes. The supernatant liquid was removed from centrifugation and successfully collected spermatozoa with good motility. Previously, spermatozoa were selected through side migration techniques to be used in ICSI procedure. Furthermore, the remaining spermatozoa that not used in ICSI are stored in a frozen liquid nitrogen tank.

On the other hand, the patient's wife underwent numbers of ovulation stimulation procedures, and ovum pick-ups carried out by an ob-gyn on infertility consultants. Obtained two eggs but after ICSI only one embryo which develops well and then transferred into the uterus of the patient's wife.

Table 1. Baseline characteristic of respondents during initial and one-month evaluation

Variables	Initial Evaluation	One-Month Evaluation
Age (Years)	42.00	-
Duration of Married (Years)	11.00	-
Semen Analysis		
Volume (ml)	2.00	5.50
Viscosity (cm)	2.00	< 2.00
Colour	Greyish White	Greyish White
Odour	Distinctive	Distinctive
pH	6.00	7.50
Concentration (million/ml)	0.10	0.15
Motility of Sperm		
Progressive (%)	10.00	34.00
Non-progressive (%)	10.00	2.00
Immotile (%)	80.00	62.00
Diagnosis	Severe OA	Severe OA
HBsAg test	Reactive	-
Anti HCV	Negative	-
Anti HIV	Non-reactive	-
FSH (mIU/ml)	42.86	-
Testosterone (ng/ml)	3.57	-
Testes Palpation Evaluation		
Volume (cc)	6.00	-
Consistency	Softness	-
Penis	Normal	-
Scrotum	Normal	-
Epididymis	Normal	-
Vas Deferens	Normal	-
Secondary Sexual Sign	Normal	-
Contraception	No	-

Evaluation on the 14th day after embryo transfer action showed no biochemical signs of pregnancy with β -hCG values of 14.35 mIU/mL and progesterone 24.82 ng/mL. However, she still gets treatment and is prepared for the second ICSI procedure.

DISCUSSION

Married couples reported that each of them was 42 years old and had problems having no offspring for 11 years. Both parties claimed that so far, they had never used contraception and had never undergone infertility therapy.

Specific physical examination of the husband found small, left and right testicular volume measuring 6 cc with a soft consistency. Added from laboratory tests found the results of semen analysis with the impression of severe OA, sex steroid hormone levels were still within normal limits, and HBsAg was positively reactive.

As we all know, HBV infection is one of the underlying problems in the world, and it was estimated that more than 75% of cases are in the Asian continent.³ A person who is chronically infected by HBV, then in saliva, vaginal fluid, semen and other body cells or tissues may be found to have HBV, in addition to blood and hepatocyte cells.⁵ This condition explains the ability of HBV to spread its infection horizontally and vertically to its offspring, especially transmission through risky sexual activity.²

In general, HBV infections are more commonly found in men; the incidence almost twice compared to women.⁷ It was found that much of the literature clearly states the relationship of chronic HBV infection with deterioration in male fertility with the occurrence of cell chromosome instability and disturbances in semen analysis especially the decrease in spermatozoa viability.^{8,9}

DNA HBV has been found in ejaculate fluids of infected patients, can be in the form of free viruses in seminal plasma or integrated into the spermatozoa genome.¹⁰ Changes in semen parameters are not so bad in men with HBV, but normal apoptosis and necrosis of spermatozoa are found, and this condition raises vertical transmission problems in the natural conception and IVF.⁹

A study found spermatozoa chromosome aberration in the HBV-infected group (14.8%), which was higher than the control (4.3%).⁵ The appearance of spermatozoa chromosomes in patients with chronic HBV infection will be thicker, clot, stain failed, and there will be a picture of specific and randomly located signal points identified by fluorescence in-situ hybridization (FISH).⁵

HBV in infertile men also underlies the infection

of other organisms, especially in genital organs, such as urealytic ureaplasma. Together with the abnormal expression of IL-17 and IL-18, urealytic ureaplasma disrupts male reproductive capacity and has a negative effect on aspects of male fertility.⁷

The small size of testis in HBV patients may be due to the occurrence of polyarteritis nodosa (PAN).¹¹ Vasculitis that attacks small arteries arises from immune complex deposition, and 7-10% of PAN cases are found with HBV infection.¹¹ The broader the provision of anti-HBV vaccines, PAN disease is increasingly rare.¹¹

Furthermore, patients were directed to IVF preparation for the first time and were given a package of Tribulus terrestris and antioxidants treatment to improve the results of semen analysis. Besides, the patient was referred to an internist to get appropriate treatment regarding HBV, to prevent vertical and horizontal transmission.

One month later, the patient and his wife underwent an IVF-ICSI procedure with the results of semen analysis having the impression of severe OA. Before the ICSI procedure was performed for spermatozoa preparation, followed by side migration technique to select functional spermatozoa, the remaining spermatozoa not used in ICSI were kept frozen. Evaluation of the 14th-day post embryo transfer (ET) did not show signs of biochemical pregnancy and the couple was planned to undergo second ICSI procedure.

At present many infertile couples with fertile chronic HBV infection want to get IVF treatment, and the number increases every year.¹² Efforts to prepare *ex-vivo* semen can reduce HBV concentration, but HBV that has been integrated into the stem cell chromosomes cannot be eliminated.⁶

A study in 2004 found a positive relationship between male and serum HBV DNA with a low ratio of implantation and pregnancy to IVF-ICSI, but not to the ratio of fertilization.⁶ On the other hand, some studies report married couples who undergo IVF-ET in men with HBV DNA positively increasing the pregnancy ratio.⁶ The latest study mentions seropositive HBsAg men have the same IVF success rate as the control group, but experience decrease in the implantation ratio and clinical pregnancy in ICSI.⁶

The possibility of vertical transmission of HBV via spermatozoa to the embryo is known to be in line with the discovery of HBV DNA integrated on the spermatozoa chromosome.¹⁰ When oocytes zones-free hamster are inseminated with human spermatozoa carrying plasmid HBV DNA, HBV was found able to replicate and express in one and two-cell embryos.¹⁰ Whereas the risk of spontaneous newborns living with HBV infection

is around 2-15%.¹³

There is still little study of the effect of HBV infection on female fertility, but a study from Lao in 2015 successfully proved the relationship between female infertility due to tubal problems with positive HBV status in male partners.⁷ HBV infection is described as able to reduce the success of IVF-ICSI using aspiration spermatozoa from the epididymis or testis.⁶

Based on the above considerations, the screening of HBV infection through a serological examination of Hepatitis-B surface antigen (HBsAg) becomes essential and mandatory for couples who will undergo IVF.⁷ Moreover, HBV infection is known to be closely related to other infectious diseases, such as hepatitis C, human immunodeficiency virus-acquired immunodeficiency syndrome (HIV-AIDS), tuberculosis and sexually transmitted infections.¹⁴

Proper handling is needed to reduce the risk of chronic HBV transmission by administering vaccines and immunoglobulin therapy.¹⁵ If one of the parties is proven to be infected by HBV, the other seronegative must be vaccinated. It was reported that 85-95% of patients undergoing vaccination therapy experience serological changes towards improvement.¹⁶

Hepatitis-B virus is known to be able to survive from direct exposure to liquid nitrogen in the frozen storage process, so it is recommended to use vapour media to prevent transmission.¹⁶ Do not forget to apply standard transmission prevention measures to medical staff who work before, throughout and after the implementation of IVF.¹⁵

CONCLUSION

This case report seeks to show various features of IVF-ICSI procedures in men with HBV and other measures that can be taken to prevent vertical and horizontal transmission.

CONFLICT OF INTEREST

Authors have declared there is no conflict of interest regarding all aspects of this study.

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No funding was received for this study which described in this article.

AUTHOR CONTRIBUTING

The first author wrote all the content of this case report manuscript and faced the patient directly under the supervision of other authors. All authors already read and approved the final manuscript.

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